

EFFECT OF CENTRALLY ADMINISTERED NITRIC OXIDE MODULATORS IN CARRAGEENIN-INDUCED NOCICEPTION IN RATS

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ABSTRACT

Possible modulation of carrageenin induced nociception by centrally (ICV) administered nitric oxide (NO) modulators was investigated. In rat, inhibitors of nitric oxide synthase inhibitors (NOS), precursor of nitric oxide (NO) with SIN-1, L-Name and haemoglobin (Hb) was explored. Nitric acid donors and scavengers are also evaluated. The pain threshold capacity is increased significantly while administration of NOS inhibitors and NO scavenger Hb whereas as hyperalgesic effect was resulted while administration of nitric oxide donors SIN-I, SNP and L-arginine. The nociceptive behavior of rat was failed to alter by D-arginine while co-administration of SIN-I with L-Name and haemoglobin found to increase the threshold level. These results showed alteration of nociceptive transmission induced by Carrageenin in rats after administration of nitric oxide modulators.

KEYWORDS: Nitric Oxide, Carrageenin, Nociception & Rats

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INTRODUCTION

Nitric oxide (NO) is a free radical gas and is formed by NO synthase (NOS) on L-arginine. NOS are widely distributed in brain as well as in peripheral tissue (Ehab *et al.*, 2012). Neuronal and endothelial NOS are two isoforms which are constantly present within body and the third isoform is must be synthesized which is not generally existing in the body. Susana *et al*, 2007 established that neuronal and endothelial NOS are present within nervous system principally while inducible NOS is largely originate in variety of cell types and macrophages, neutrophils and chondrocytes. In pain model study, NO is a key mediator of nociceptive activity in various animal.

Studies have suggested a role of NO, a second messenger in nociceptive reflexes as well as in modification of anti-nociception induced by morphine (Jadav *et al.*, 2000). L-arginine and NO donors such as sodium nitroprusside (SNP), nitroglycerin and 3- morpholinosydnonimine (SIN-I) have been demonstrated to exhibit anti-nociceptive activity in rats with carrageenin-induced hyperalgesia (Jose *et al.*, 2002). Interestingly L-arginine and NO donors enhance nociception or inflammatory response elicited by bradykinin, substance P, carrageenan and dextran while L-name suppresses the activities. Thus, there exists evidence of both nociceptive and anti-nociceptive role of NO in peripheral tissues. Further, peripherally or centrally administered NOS

inhibitors have been shown to produce anti-nociceptive effect in mice (B L *et. al.*, 2001). NO found in the central nervous system plays an important role in the central processing of painful stimuli. Although, there is considerable evidence implicating NO in Brewer's yeast, formalin and bradykinin-induced nociception (Jadav *et. al.*, 2000; Adzu *et. al.*, 2003; Sophia *et. al.*, 2011), but there is no much information on carragennin induced nociception. Hence, the present study has been undertaken to investigate ICV administered isoform, selective and non-selective inhibitors of NOS and other NO modulators in Carrageenin-induced nociception in rats.

MATERIALS AND METHODS

The studies were conducted on adult male Wistar rats (175-225 g). The animals (6 in each group) were housed individually with free access to clean drinking water and balanced feed. Trials were conducted at an atmospheric temperature range of $25 \pm 2^\circ\text{C}$ between 9 am to 4 pm. Intra Cerebro Ventricular (ICV) cannulation was executed by embedding a polyethylene cannula (No. 47) into the right lateral ventricles by injecting intramuscularly anesthetic ketamine hydrochloride @ dose of 100 mg/kg. The operated rats were permitted to recover for 7 days before starting experiments. Apart from co-administration of SIN-1 of dosage 500 μg with L-NAME of 100 μg and Hb of 200 μg , test drugs administered ICV route in rats included, 100 μg each N-Nitro-Larginine methyl ester, L-N-monomethylarginine, 7-nitroindazole and L-N iminoethyl lysine, while 50 μg of L-arginine and D-arginine. 500 μg of 3- morpholinysydnonimine and 250 μg of sodium nitroprusside and bovine hemoglobin and 20 μg methylene blue were administered respectively. Co-administration of SIN-1 (500 μg) with L-NAME (100 μg) and Hb (200 μg) was also given.

Drugs were administered to rats by ICV 30 min prior to induction of nociception by Carrageenin. Except 7-NI which was dissolved in arachis oil, drugs obtained from Sigma Chemical were dissolved in artificial CSF. 10 μl micro syringes are utilized for injection of ICV of a constant volume of 5 μl . Artificial CSF or arachis oil were received by control animals in equal volume.

Rats received an intra-planter injection of 0.1 ml of 1% carrageenin in CMC (carboxy methyl cellulose) solution in the right hind paw. The pressure in the Carrageenin injected paw was recorded as pain threshold by Randali-Selitto assay immediately prior to and at 1, 3 and 5 hr post- Carrageenin injection. In brief, pain threshold was measured by applying pressure to inflamed paw at steadily increasing rate by means of pedal switch of Randali-Selitto apparatus (UGO Basile, Italy). The end point or "Pain threshold" is the pressure necessary to cause animals to struggle. The change in pain threshold in test groups was compared with that of untreated control group.

For ascertaining the correct position of cannula in the ventricles after termination of the experiments, all rats were administered 5 μl of 1% Evans blue dye solution ICV and brain was removed, sectioned and examined.

Statistical analysis of the data was performed using Student's *t* test (SPSS 19.0 version) to study the difference amongst the mean values.

RESULTS

Table 1: Effect of Centrally Administered NOS Inhibitors and NO Precursor on Carrageenin-Induced Nociception in Rats [Values are Mean $\pm$ S. E. from 6 Animals in Each Group]

NOS Inhibitor	Dose (μ g)	Pressure on Paw in g (Pain Threshold, Mean $\pm$ S.E.)			
		0 h	1 h	3 h	5 h
Control	-----	77.5 $\pm$ 3.8	53.3 $\pm$ 3.3	49.2 $\pm$ 3.0	41.6 $\pm$ 4.5
L-NAME	50	75.0 $\pm$ 4.1	69.2 $\pm$ 8.3	86.7 $\pm$ 6.3***	98.3 $\pm$ 5.4***
	100	77.5 $\pm$ 7.1	77.5 $\pm$ 5.4**	97.5 $\pm$ 5.3***	118.3 $\pm$ 6.5***
	200	83.3 $\pm$ 7.0	109.1 $\pm$ 10.9***	147.5 $\pm$ 10.6***	174.1 $\pm$ 10.9***
L-NMMA	50	75.8 $\pm$ 7.0	67.5 $\pm$ 8.2	70.8 $\pm$ 4.1**	91.6 $\pm$ 6.0***
	100	73.4 $\pm$ 6.5	73.3 $\pm$ 5.3*	74.1 $\pm$ 5.2**	116.6 $\pm$ 9.8***
	200	75.0 $\pm$ 4.4	71.6 $\pm$ 3.3**	105.8 $\pm$ 12.5**	146.6 $\pm$ 12.8***
7-NI	50	68.3 $\pm$ 6.9	66.6 $\pm$ 3.1*	104.2 $\pm$ 12.3**	123.3 $\pm$ 8.1***
	100	77.5 $\pm$ 8.9	76.6 $\pm$ 8.3*	111.6 $\pm$ 12.6***	142.5 $\pm$ 13.6***
	200	70.0 $\pm$ 4.6	83.3 $\pm$ 8.9*	112.5 $\pm$ 9.6***	148.3 $\pm$ 11.9***
L-NIL	50	76.6 $\pm$ 9.3	53.3 $\pm$ 6.1	74.2 $\pm$ 7.9*	100.0 $\pm$ 8.8***
	100	70.0 $\pm$ 8.1	70.8 $\pm$ 6.6*	75.8 $\pm$ 9.1*	100.8 $\pm$ 7.7***
	200	75.8 $\pm$ 7.2	103.3 $\pm$ 6.1***	100.8 $\pm$ 9.4***	120.0 $\pm$ 9.6***
NO Precursor					
Control	-----	86.6 $\pm$ 7.2	61.6 $\pm$ 5.4	59.1 $\pm$ 5.5	49.1 $\pm$ 4.9
L-arginine	50	75.0 $\pm$ 8.9	65.0 $\pm$ 8.2	60.0 $\pm$ 5.9	56.6 $\pm$ 5.7
	100	85.0 $\pm$ 7.1	51.6 $\pm$ 4.6	37.5 $\pm$ 4.4*	33.3 $\pm$ 2.7*
	200	80.8 $\pm$ 6.6	42.5 $\pm$ 5.7*	36.6 $\pm$ 4.6*	30.8 $\pm$ 3.9*
D-arginine	200	77.5 $\pm$ 8.3	58.3 $\pm$ 4.9	59.1 $\pm$ 7.2	55.8 $\pm$ 8.8

n=6 (Six) animals in each group; *p<0.05, **p<0.01, ***p<0.001 when compared with the controls; 0 h: before injection of carrageenan

Table 2: Effect of Centrally Administered NOS Inhibitors and NO Precursor on Carrageenin-Induced Nociception in Rats [Values are Mean $\pm$ S. E. from 6 Animals in Each Group]

NO Modulators	Dose (µg)	Pain Threshold (g), Mean ± S.E.			
		0 h	1 h	3 h	5 h
NO Donors					
Control	-----	90.0 ± 4.1	66.6 ± 4.9	55.8 ± 5.3	47.5 ± 3.8
SNP	5	96.6 ± 3.8	70.0 ± 5.3	50.8 ± 4.5	44.1 ± 5.1
	10	88.3 ± 5.2	67.1 ± 6.1	50.8 ± 5.9	31.6 ± 3.8*
	20	92.5 ± 3.8	59.1 ± 5.1	41.6 ± 3.5*	32.5 ± 3.8*
SIN-1	125	95.8 ± 4.7	65.8 ± 5.4	53.3 ± 4.4	32.5 ± 3.1*
	250	85.0 ± 2.8	62.5 ± 5.4	40.0 ± 5.3*	28.3 ± 4.2**
	500	98.3 ± 5.1	65.8 ± 4.9	37.5 ± 4.2*	27.5 ± 3.5**
NO Scavengers					
Control	-----	93.3 ± 4.7	66.6 ± 5.5	51.6 ± 4.4	45.0 ± 5.3
Hemoglobin	100	90.0 ± 4.2	69.1 ± 5.0	60.8 ± 6.2	68.3± 4.4**
	200	100.0 ± 4.8	74.1 ± 5.5	85.8 ± 3.9***	101.6 ± 6.9***
	400	91.6 ± 5.7	84.1 ± 5.5*	100.0 ± 5.6***	100.8± 5.6***
Methylene blue	25	95.8 ± 4.7	62.5 ± 4.4	52.5 ± 4.6	47.5 ± 6.1
	50	102.5 ± 5.8	64.1 ± 6.8	59.1 ± 5.6	46.6 ± 3.8
	100	93.3 ± 5.6	73.3 ± 4.9	54.2 ± 4.7	52.5 ± 6.3
Other Combinations					
Control	-----	90.0 ± 5.7	58.8 ± 5.1	40.8 ± 6.2	30.8 ± 4.3
L-arginine+L-NAME	100+100	94.1 ± 5.5	77.5 ± 6.4*	95.8 ± 7.8***	100.0 ± 7.3***
SIN-1 + L-NAME	500+100	95.8 ± 4.3	55.8 ± 5.6	43.3 ± 4.9	19.1 ± 2.7**
SIN-1 + Hemoglobin	500 +200	85.0 ± 5.0	75.0 ± 5.7*	91.1 ± 4.9***	90.8 ± 6.1***

n=6 (Six) animals in each group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with the controls; 0 h: before injection of carrageenan

The data on pain threshold following ICV administration of NOS inhibitors in carrageenan induced noniception are summarized in Table 1. In control group, the pain threshold at 0 hr (before carrageenan administration) was 77.50 ± 3.82 g, which was significantly reduced to 53.33 ± 3.33 , 49.17 ± 3.00 and 41.67 ± 4.59 g at 1, 3 and 5 hr, respectively. Central administration of L-NAME and L-NMMA showed significant increase in pain threshold at 3 to 5 h (50 μ g) from 1 to 5 hr (100 μ g) and 1 to 5 h (200 μ g). 7 NI showed its inhibitory effect from 1 to 5 hr at all doses. The iNOS inhibitor, L-NIL was found to be effective in increasing the pain threshold from 3 to 5 h with 50 μ g and 1 to 5 h with 100 μ g and 1 to 5 h with 200 μ g.

The maximum nociception response following carrageenan injection in control group rats was observed at 5h (47.50 ± 3.82 g) (Table 2). SNP at the dose of 5 μ g failed to alter the nociception behavior, whereas at the dose of 10 and 20 μ g, the nociception was enhanced by reducing the pain threshold at 5 hr (10 μ g) and from 3 to 5 h (20 μ g) respectively. ICV administration of SIN-1 was found to be hyperalgesic, being effective at 5 h (125 μ g) and from 3 to 5 h (250 and 500 μ g).

The mean pain threshold in control rats varied from 66.67 ± 5.58 g at 1 hr to 45.00 ± 5.32 g at 5 h post-carrageenin injection. Hemoglobin increased the pain threshold capacity in dose-dependent manner, 100 μ g dose being effective at 5h, 200 μ g from 3 to 5 h and 400 μ g from 1 to 5 h, while methylene blue (25, 50 and 100 μ g) had no effect on pain threshold (Table 2).

The result of combined ICV administration of NO modulators on pain threshold are presented in Table 2. The combination of L-arginine (100 μ g) with L-NAME (100 μ g) significantly increased pain threshold, with SIN-1 (500 μ g) and L-NAME (100 μ g) at only 5 h. The co-administered SIN-1 (500 μ g) with hemoglobin (200 μ g) was antinociceptive from 1 h to 5 h.

DISCUSSIONS

The choice of carrageen in was based on the production of acute inflammation and concomitant strong hyperalgesia (Jurgen Sandkuhler, 2009). Oedema production may involve production of NO, since NOS synthase inhibitors (L-NAME, L-NMMA, 7 NI and L-NIL) and haemoglobin (Hb), a scavenger of NO, were potent inhibitors of paw oedema volume in the late phase (1 – 5 h) than early phase (15-60 min) of inflammation (Giuseppe *et al.*, 2006). The non-specific NOS (eNOS and iNOS) inhibitors (L-NAME and L-NMMA), n NOS inhibitor (7 – NI) and iNOS inhibitor (L-NIL) when given by ICV, 30 mins prior to onset of inflammation, attenuated the oedema at sustained phase indicating involvement of endogenous process. This result suggests the release of endogenous NO in acute inflammation which modulates oedema formation in carageenin induced peripheral inflammation. NO may be generated either by eNOS and / or iNOS or both in dextran and carrageenin induced oedema formation (Ialenti *et al.*, 1993; Inmaculada *et al.*, 2004). Similar results have been reported in a study on the regulatory role of endogenous NO in the oedema formation induced in rats by substance P (Hughes *et al.*, 1990) ICV administered of L-NAME reduced formalin induced paw licking time, suggesting an effect within the central nervous system (Sophia *et al.*, 2011). L- NAME is antinociceptive in hotplate assay, which is believed to be sensitive solely to drugs acting supra-spinally (TL Yaksh 1997). Moore and co-workers reported that intra-peritoneal injection of the nNOS inhibitor (7 – NI), significantly reduced the hind paw licking behavior in the

formalin induced inflammation (Moore *et. al.*, 1993). Intrathecal (i.t.) administration of iNOS selective inhibitor aminoguanine was able to inhibit thermal but not mechanical hyperalgesia in zymogen induced inflammation model (Chizuko *et. al.*, 2003).

The NO precursor, L-arginine produced hyperalgesia from 1 hr onwards to 5 hr observation period significantly with an ICV dose of 200 µg, but its isomer, D-arginine (200 µg) was without any effect. This suggests that D-isomer of L-arginine is inactive. L-arginine is reported to enhance nociception produced carragenen (Ialenti *et. al.*, 1993) and reduced nociception in carragenin induced hyperalgesia (Abbasivash *et. al.*, 2009). The antinociceptive effect of L-arginine was attributed to the formation of kyotorphin in brain (Atsufumi *et. al.*, 1996), an antinociceptive dipeptide. However, chronic treatment with L-arginine produces selective increase (15%) in the activity of NOS in the mid brain. The increase in NOS activity would enhance production of NO and studied showed NO to have an algesic action (Ana *et. al.*, 2015). Therefore increase production of NO as a result of administration of L-arginine may have resulted in observed in hyperalgesic effect of L-arginine in the present study.

The NO donors, SNP and SIN-1 reduced the pain threshold significantly from 3 h post –injection of inflammogens in the present study. Similar hyperalgesic response of NO donors was also reported by Bhargava *et. al.*, 1997 and Megan *et. al.*, 2014 when administered by intrathecal route and into the dorsal horn, respectively. SNP was also reported to increase cGMP level in dorsal route ganglia satellite cells in young rats (Shi *et. al.*, 1998).

Haemoglobin (Hb), a scavenger of NO, was antinociceptive as it increased the pain threshold significantly in dose-dependent manner in the present investigation. The highest dose of Hb (400µg) potentiated the pain threshold throughout the observation period of 5h. Methylene blue did not modify the nociceptive response at all the three dose levels (25, 50 and 100µg) used in the present study, suggesting that endogenous NO modulates carrageenan by a cGMP independent mechanism. The observations are in agreement with earlier reports of Xu and Tseng (Xu *et. al.*, 1994; H Machelska *et. al.*, 1999) that Methylene blue failed to alter the tail-flick nociception on icv administration and did not affect the base line threshold.

In the present study, co-administration of L-arginine with L-NAME partially reversed the suppressive effect of L-NAME on pain threshold capacity. Similar partial antagonistic effect of NO precursor L-arginine on antinociceptive responses of NOS inhibitors (7-NI, L-NAME and L-NMMA) have been reported earlier (Moore *et. al.*, 1994; Wiliam *et. al.*, 2002).

CONCLUSIONS

Simultaneous ICV administration of SIN-1 and L-NAME reduce pain threshold significantly only at 5 h after inducing nociception. This observation in the present study further confirms the participation of exogenously applied from NO donor, SIN-1, in nociceptive processing in central nervous system, while endogenously generated NO was blocked by NOS inhibitor, L-NAME. Administration of Hb in combination with SIN-1 showed significant antinociceptive effect suggesting that Hb is an effective molecule in binding and inactivating both exogenously applied or endogenously generated NO.

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